# Supplementary Information

# High Resolution Data Independent Acquisition Electron Transfer Dissociation Mass Spectrometry: Multiplexed Analysis of Post-translationally Modified Proteins

*Michael J. Sweredoski[1], Tonya Pekar Second[2], Jenny Broeker[3], Annie Moradian[1], and Sonja Hess[1,*]*

[1]Proteome Exploration Laboratory, Division of Biology, Beckman Institute, California Institute of Technology, Pasadena, CA 91125, USA

[2]Thermo Scientific, San Jose, CA 95134, USA

[3] Institute of Pharmacology, University of Heidelberg, Heidelberg 69120, Germany

S1

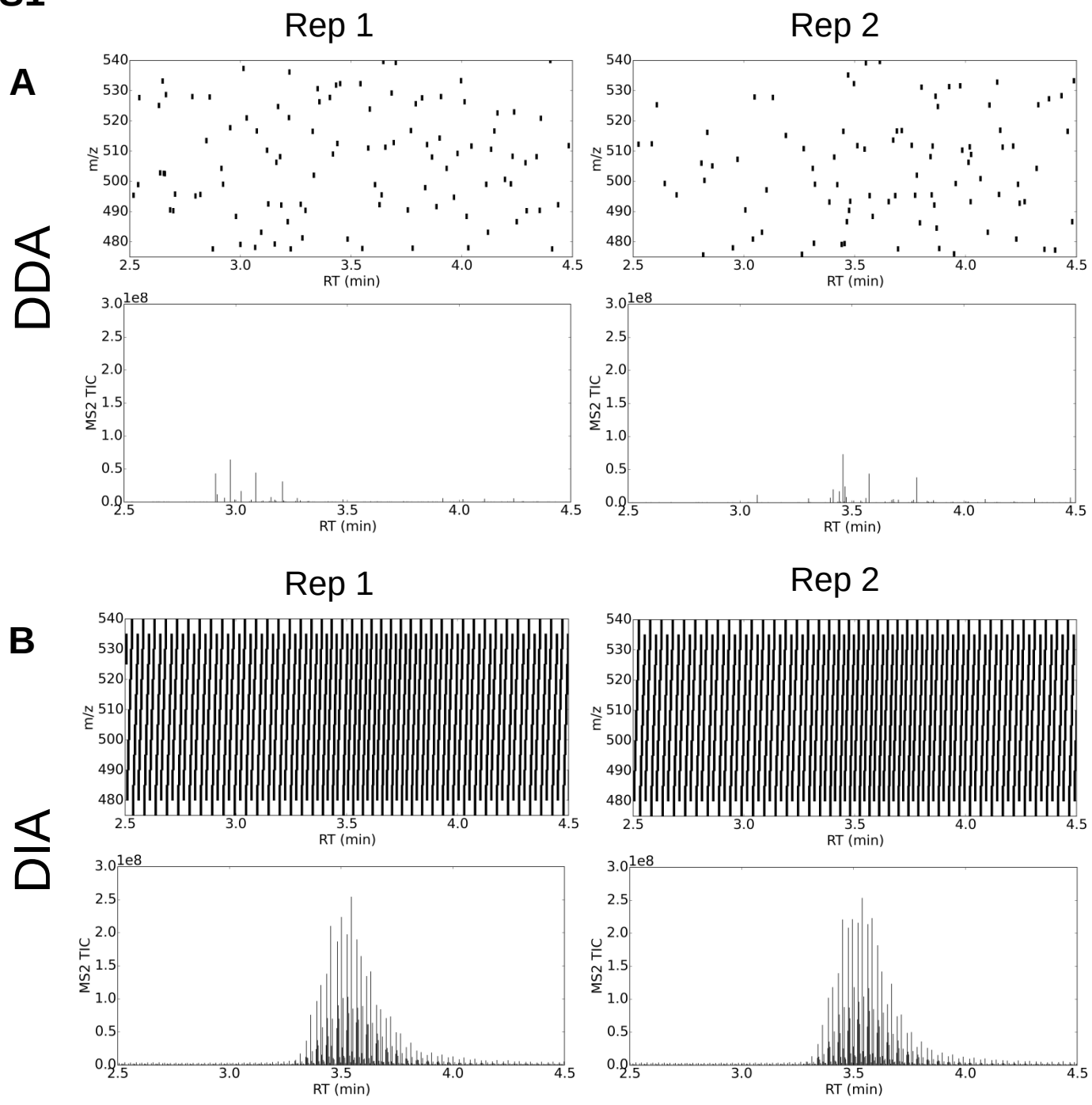
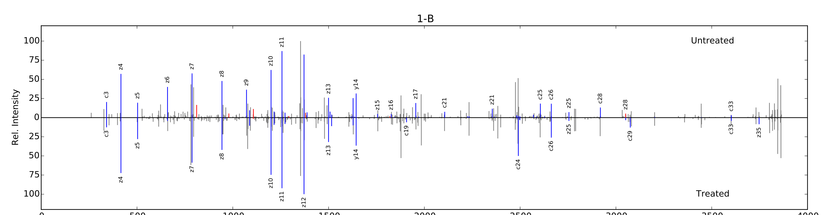
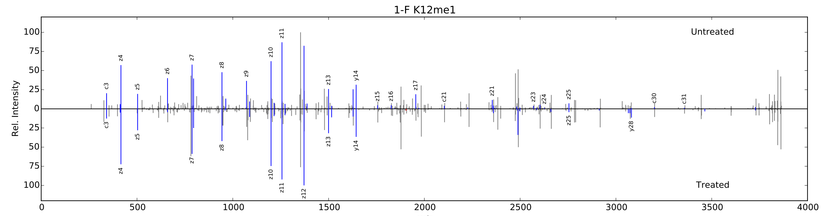
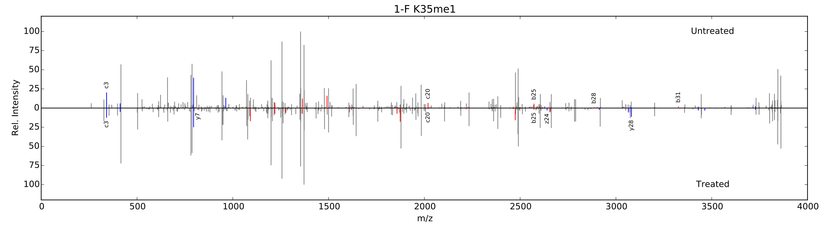
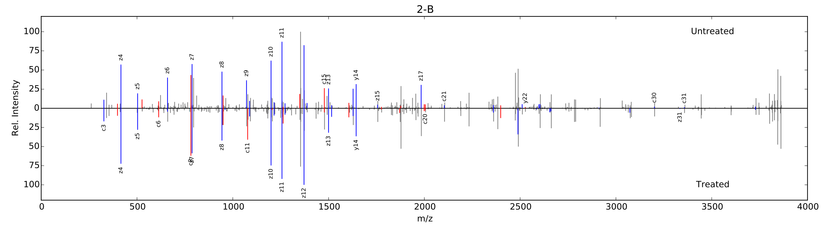


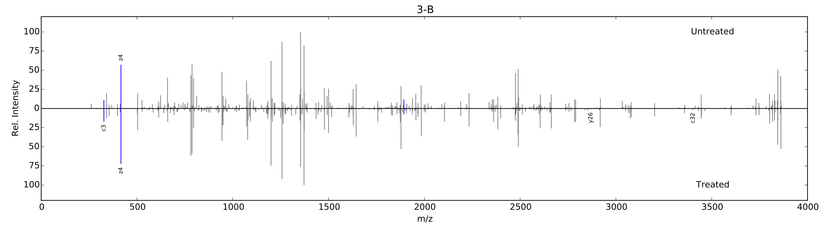
**Figure S1:** Shown are two replicate analyses in (A) DDA ETD and (B) DIA ETD mode. (A) DDA shows sporadic sampling over the selected mass range (top) resulting in unreliable MS2 total ion chromatograms that vary significantly from run to run (bottom, compare left and right). The bars in the top panel represent actual isolation windows at given retention times. (B) DIA shows predictable and reliable sampling over the selected mass range (top) resulting in reproducible MS2 total ion chromatograms that are identical from run to run (bottom, compare left and right). The bars in the top panel represent actual isolation windows at given retention times.

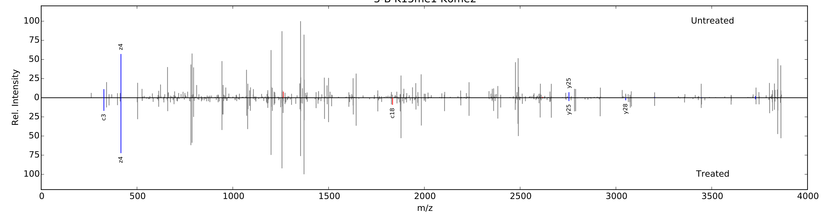

1-B
Untreated
Treated
Rel. Intensity
m/z

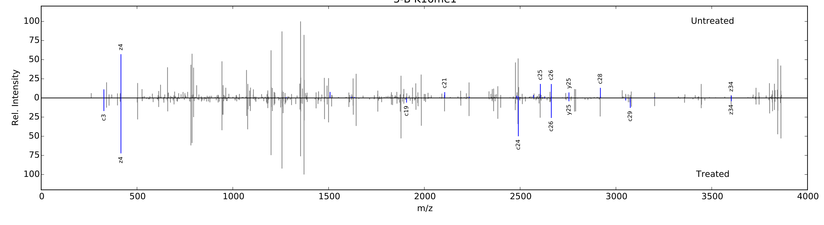

1-F K12me1
Untreated
Treated
Rel. Intensity
m/z

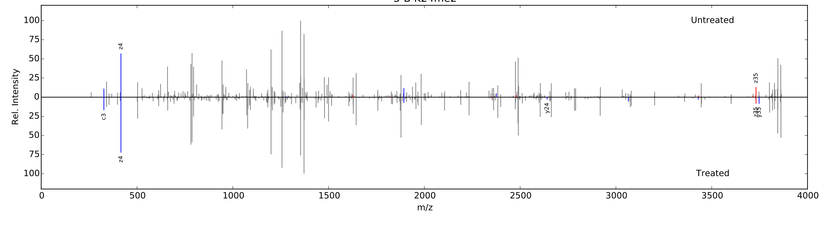

1-F K35me1
Untreated
Treated
Rel. Intensity
m/z

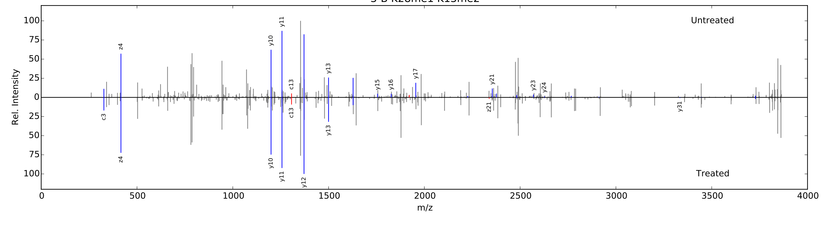

2-B
Untreated
Treated
Rel. Intensity
m/z


3-B
Untreated
Treated
Rel. Intensity
m/z


3-B K13me1 K6me2
Untreated
Treated
Rel. Intensity
m/z


3-B K16me1
Untreated
Treated
Rel. Intensity
m/z


3-B K24me2
Untreated
Treated
Rel. Intensity
m/z


3-B K28me1 K13me2
Untreated
Treated
Rel. Intensity
m/z

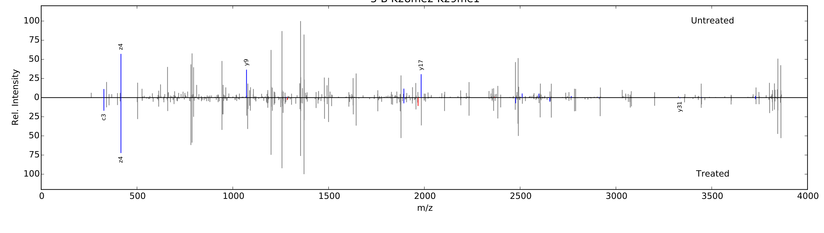

3-B K28me2 K29me1
Untreated
Treated
Rel. Intensity
m/z

**Figure S2:** Displayed are the fragment ion matches of the minimum set of proteoforms (1-B, 1F, K12me1, 1F K35me1, 2-B, 3-B, 3-B K13me1 K6me2, 3-B K16me1, 3-B K24me2, 3-B K28me1 K13me2, and 3-B K28me2 K29me1) for the 475-485 precursor ion windows of the untreated and treated samples. Ions highlighted in blue are shared by multiple proteoforms, ions highlighted in red are unique to a particular proteoform. H2B3BK16me1 was a proteoform that was deduced to be present because of its precursor ions indicating a methylated H2B3B.

| # Explained Fragment Ions | Replicate 1 | Replicate 2 |
|---|---|---|
| **CID** | 37 | 30 |
| **ETD** | 115 | 117 |

**Table S1:** Comparison of CID and ETD in DIA mode shows ETD is superior to CID in the number of explained fragment ions. ETD produced approximately three times more fragment ions that could be assigned to the H2B proteoforms present. This difference was significant with a p-value of 0.002.